What can we learn from sequencing mycetoma fungi?

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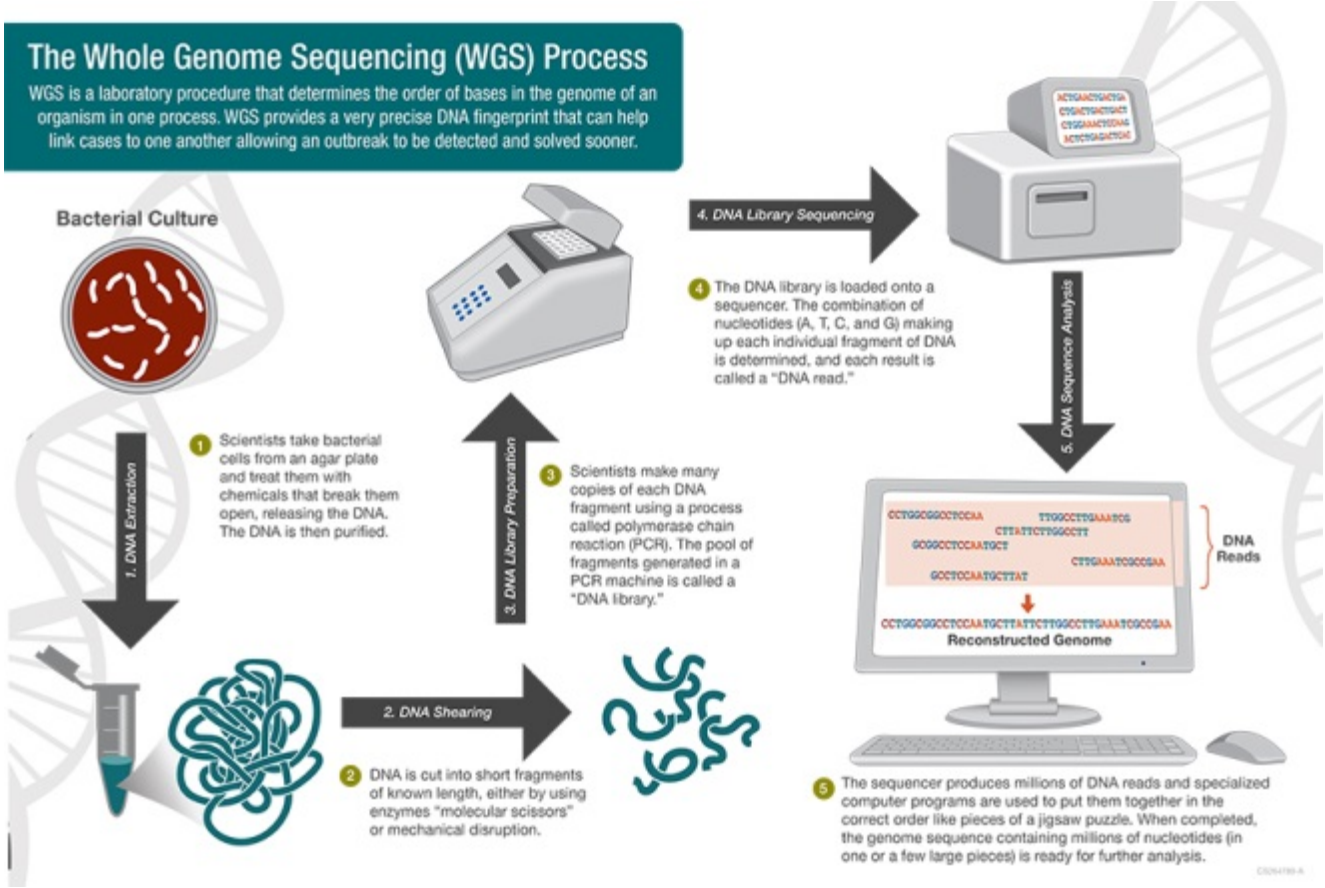
11th ECTMIH, Liverpool 2019


The Whole Genome Sequencing (WGS) Process
WGS is a laboratory procedure that determines the order of bases in the genome of an organism in one process. WGS provides a very precise DNA fingerprint that can help link cases to one another allowing an outbreak to be detected and solved sooner.
Bacterial Culture
1. DNA Extraction
1 Scientists take bacterial cells from an agar plate and treat them with chemicals that break them open, releasing the DNA. The DNA is then purified.
2. DNA Shearing
2 DNA is cut into short fragments of known length, either by using enzymes "molecular scissors" or mechanical disruption.
3. DNA Library Preparation
3 Scientists make many copies of each DNA fragment using a process called polymerase chain reaction (PCR). The pool of fragments generated in a PCR machine is called a "DNA library."
4. DNA Library Sequencing
4 The DNA library is loaded onto a sequencer. The combination of nucleotides (A, T, C, and G) making up each individual fragment of DNA is determined, and each result is called a "DNA read."
5. DNA Sequence Analysis
DNA Reads
Reconstructed Genome
5 The sequencer produces millions of DNA reads and specialized computer programs are used to put them together in the correct order like pieces of a jigsaw puzzle. When completed, the genome sequence containing millions of nucleotides (in one or a few large pieces) is ready for further analysis.


PHASE TWO: INTERPRETATION
I THINK I FOUND A CORNER PIECE.
3 BILLION PIECES
GENOME

Advantages of WGS for mycetoma community

- Better understanding of etiology of mycetoma
- Identification of novel targets for new diagnostics methods

Understanding etiology of mycetoma: better species identification

Molecular methods based on a single gene do not always provide enough resolution for identification of species

TABLE 2 Phenotypic and molecular data from eumycetoma agents

Case	Morphological identification	Molecular identification	ITS GenBank accession number This study/Reference	ITS Identity	D1/D2 GenBank accession number This study/Reference	D1/D2 Identity
1	*Madurella mycetomatis*	*Madurella pseudomycetomatis*	KT834405/EU815933	596/597 (99%)	KX580969/EF600939	579/580 (99%)
2	*Exophiala jeanselmei*	*Cyphellophora oxyspora*	KT323976/KM396285	600/602 (99%)	KX580971/KF928530	435/436 (99%)
3	*Exophiala* sp.	*Exophiala oligosperma*	KT323978/DQ836792	655/655 (100%)	KX580972/KP938217	609/609 (100%)
4	*Exophiala dermatitidis*	*Exophiala dermatitidis*	KT323977/AY213651	657/657 (100%)	KX580974/AF050270	615/618 (99%)
5	*Scedosporium apiospermum*	*Scedosporium apiospermum*	KT323975/AB489076	636/639 (99%)	KX580973/FJ345358	380/382 (99%)
6	*Aspergillus ustus*	*Aspergillus ustus*	KT323974/EU326214	590/595 (99%)	KX580970/AY216676	594/595 (99%)

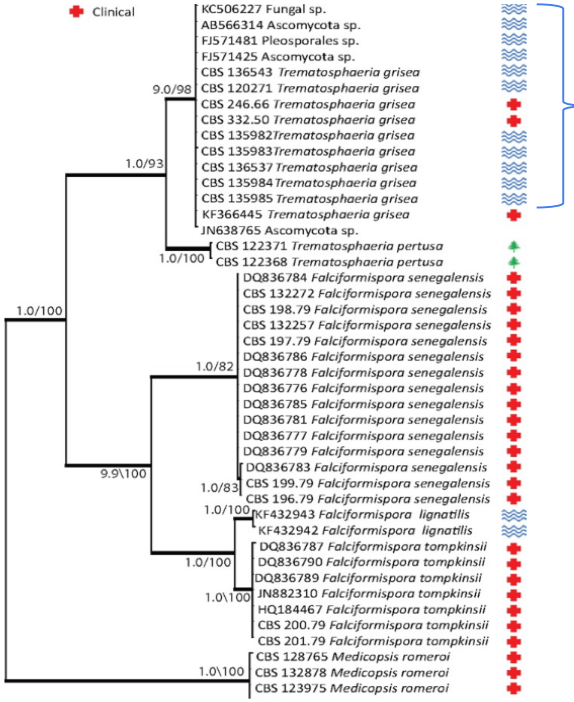
TABLE 3 Phenotypic and molecular identification data from actinomycetoma agents

	16S rDNA

Require different genes for identification

Rojas et al, 2016

Understanding etiology: diversity within species



ITS gene (values of ≥ 0.8 for Bayesian probability and ≥ 80 % for maximum likelihood are shown with **bold** branches). *Medicopsis romeroi* v

Trematosphaeria grisea

Ahmed et al, 2014

Novel diagnostics

Ideal molecular target for DNA-based detection:

- Specific for mycetoma agents (does not cross-react with other soil fungi)
- Shared by different species/genera (*Madurella mycetomatis* and *Trematosphaeria grisea*)
- Present in multiple copies to increase sensitivity

This approach worked well for another fungus

AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology

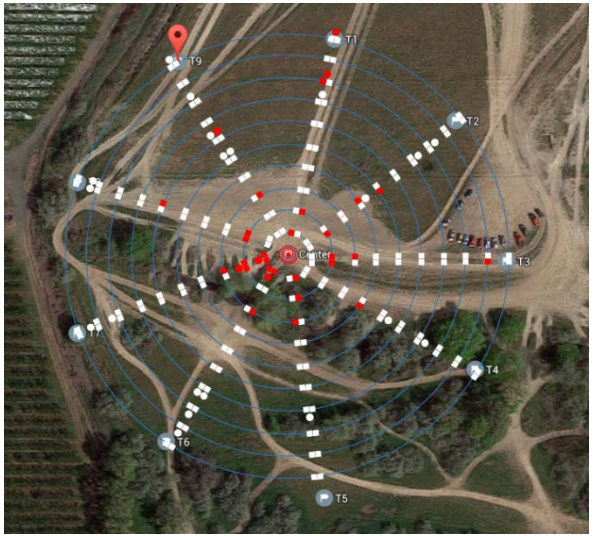
Mycology

Multicenter Clinical Validation of a Cartridge-Based Real-Time PCR System for Detection of *Coccidioides* spp. in Lower Respiratory Specimens

Michael A. Saubolle, Bette R. Wojacki, Anne M. Wertheimer, Atshkeng Z. Fuseyagem, Stephen Young, Brian A. Koeneman
David W. Warnock, Editor

DOI: 10.1128/JCM.01277-17

Article Figures & Data Info & Metrics PDF



AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

Eukaryotes

Genome Sequence of *Madurella mycetomatis* mm55, Isolated from a Human Mycetoma Case in Sudan

Sandra Smit, Martijn F. L. Derks, Sander Bervoets, Ahmed Fahal, Willem van Leeuwen, Alex van Belkum, Wendy W. J. van de Sande

DOI: 10.1128/genomeA.00418-16

36.7Mbp genome
804 scaffolds (N50 of 81.8 kb; G+C content of 54.9%).

Collaboration between Mycetoma Research Center, Sudan and CDC

Whole Genome Sequencing of fungal agents of Mycetoma

Study objectives

- Generate chromosomal quality annotated genomic assemblies of *M. mycetomatis* and *T. grisea* using long-read sequencing --- to provide a resource for community

- Generate WGS phylogeny of *M. mycetomatis* using clinical isolates from Sudan --- to understand the genetic diversity among isolates

- Use metagenomics to characterize “grains” from mycetoma patients--- to understand what pathogens actually are present in patients

Study Samples

- **Received from Prof. Fahal's group:**
    - 128 DNA from grains
    - 50 cultures of *M. mycetomatis*
- **Two isolates (one *M. mycetomatis* and one *T. grisea*) from CDC collection**

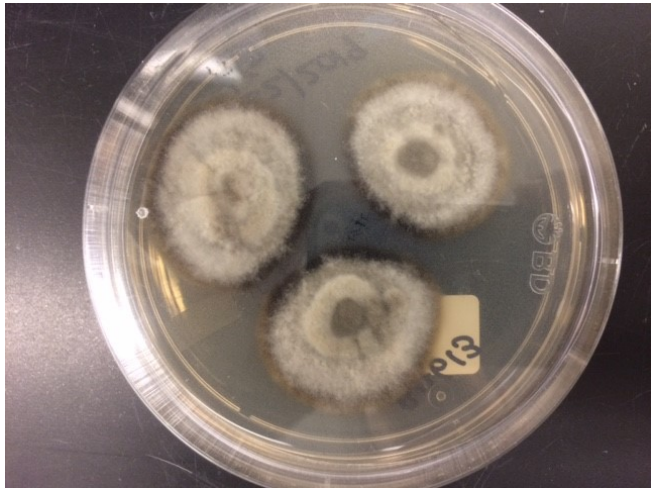
Preliminary PCR analysis of grain samples (ITS and 16S)

Organism	no 16S amplification	Actinomadura sp.	Uncultured/ unsequenced	*S. pyogenes*
M. mycetomatis	**92**	0	19	2
M. fahalii	1	0	0	0
Falciformispora thompkinsii	1	0	0	0
Falciformispora senegalensis	1	0	0	0
Cladosporium sp.	2	0	0	0
Curvularia sp.	1	0	0	0
Fusarium solani	1	0	0	0
no ITS amplification	4	**7**	5	0

Of 126, 88 passed DNA quality control for WGS and good quality reads were obtained – analysis pending

Cultures

- Of 50, 29 cultures grew
- 26 were sent for WGS
- 3 are slow growing



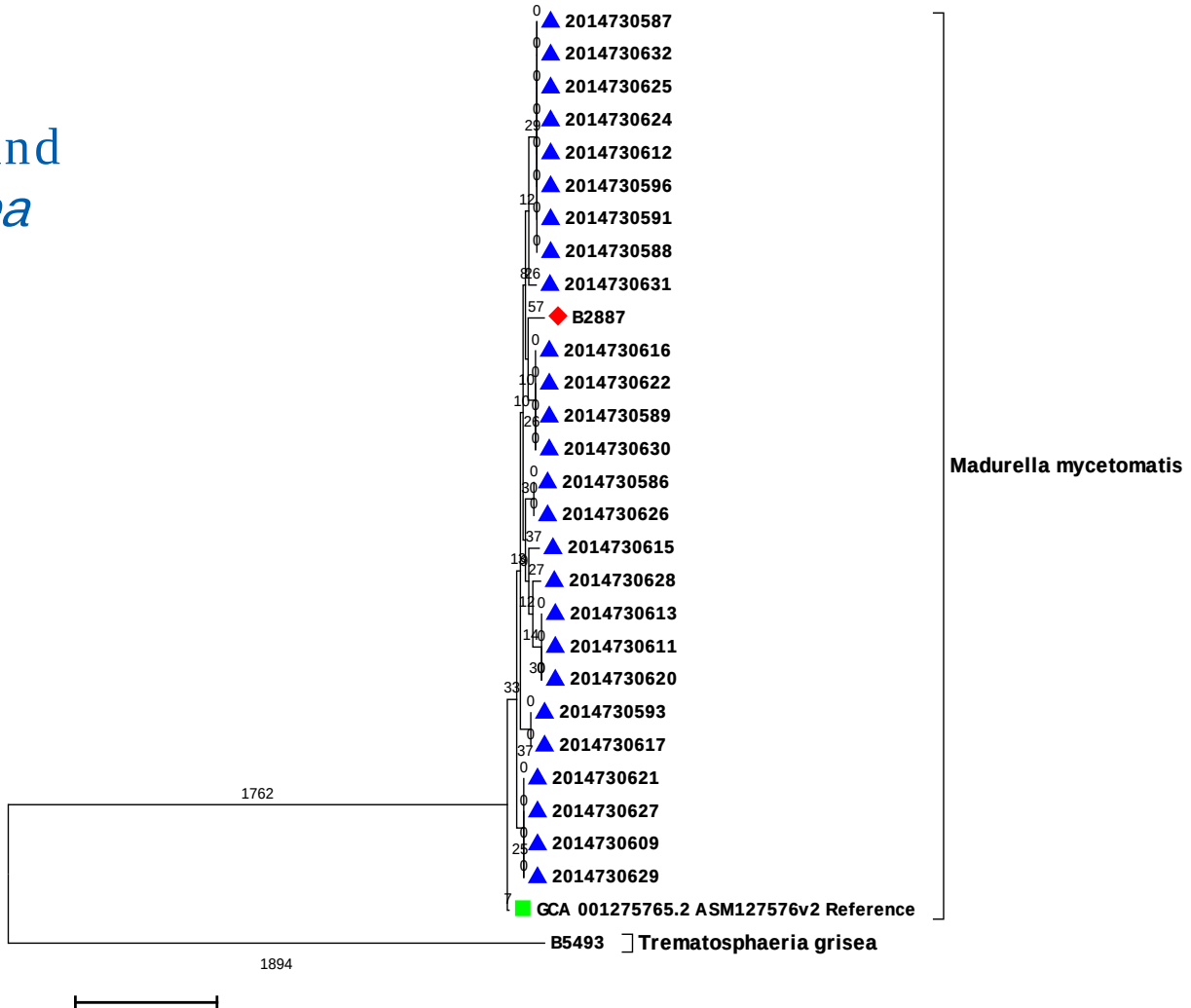
T. grisea

M. mycetomatis

Preliminary WGS results

Madurella mycetomatis and *Trematosphaeria grisea*

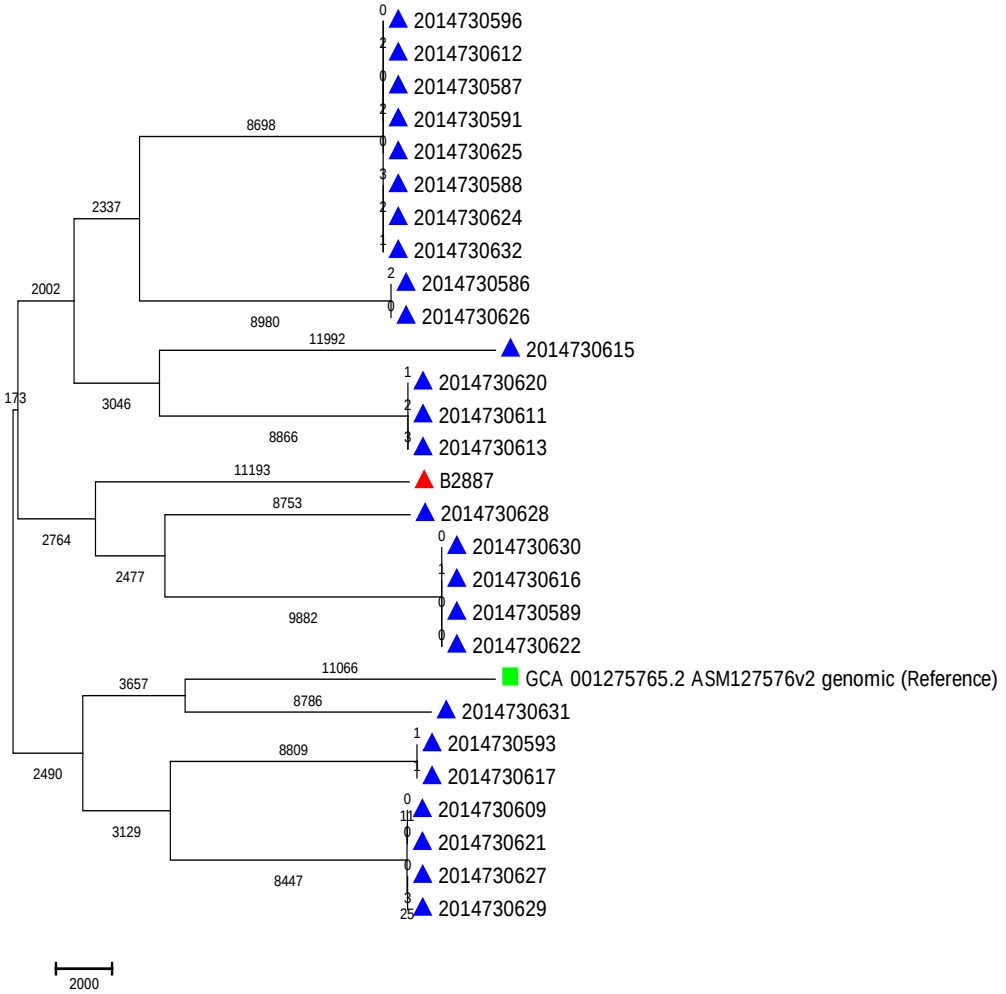
- Isolates from Sudan
- CDC Collection
- NCBI Reference



Preliminary WGS results

M. mycetomatis only

- Isolates from Sudan
- CDC Collection
- NCBI Reference



Next steps

- PacBio sequencing of 5 isolates, *T. grisea* and four *M. mycetomatis*
- Long-read assembly and annotation
- WGS phylogeny of *M. mycetomatis*
- Identification of potential PCR targets
- Collaboration of developing molecular tests
- WGS of isolates from other regions and other genera?

Acknowledgments

Mycetoma Research Center, Sudan

Prof. Ahmed Fahal
Sahar Bakhiet

Mycotic Diseases Branch, CDC

Lalitha Gade
Steven Hurst
Karlyn Beer
Tom Chiller

For more information, contact CDC
1-800-CDC-INFO (232-4636)
TTY: 1-888-232-6348 www.cdc.gov

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.